REMARKS

Applicant respectfully requests reconsideration of the above-identified patent application in view of the amendment above and the remarks below.

Claim 7 has been canceled in this paper. Claim 1 has been amended in this paper. No new claims have been added in this paper. Therefore, claims 1-6 and 8-14 are pending and are under active consideration.

Claim 2 stands rejected under 35 U.S.C. 112, second paragraph, "as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention." In support of the rejection, the Patent Office states the following:

> Claim 2 is indefinite for reciting the phrase, "[t]he method according to claim 1...whereby the immobilized oligomers hybridize at least one of the primers used in the amplification step."
>
> There is insufficient antecedent basis for the term, "primers" in neither claim 2 nor in parent claim 1.

Applicant has amended claim 1 to provide, via inherency, antecedent basis for the term "primers" in claim 2. Accordingly, the subject rejection has been overcome and should be withdrawn.

Claims 1-3, 6-10, 12 and 13 stand rejected under 35 USC 103(a) "as being unpatentable over Gonzalgo et al. (WO 98/56952, published December 17, 1998) in view of Yurov et al. (Human Genetics, 1996, vol. 97, pages 390-398) and in light of Davis et al. (U.S. Patent No. 6,046,002, issued April 4, 2000, filed January 5, 1998), made in the Office Action mailed on March 1, 2005...for the reasons of record." In support of the rejection, the Patent Office states the following:

> Applicants' arguments presented in the Amendment received on August 24, 2005 have been fully considered but they are not found persuasive for the following reasons.

The Rejection:

Gonzalgo et al. disclose a method of fluorescently detecting the methylated cytosine in a genomic DNA sample, wherein the genomic DNA is first treated with a bisulfite (page 4, line 15; claim limitation 6), the DNA amplified by PCR or polymerase chain reaction, incorporating radioactively labeled dNTPs, such as dCTP and dGTP (page 7-8; claim limitation 7), amplicons separated via electrophoresis (page 5, line 24; claim limitation 3), and the amplicons detected radioactively (page 4, lines 10-30) or fluorescently (page 8, lines 30-31; claim limitation 12). Gonzalgo et al. also disclose a method of detecting the methylated cytosine, wherein the amplicons are transferred onto a nylon membrane for dot-blot analysis (page 8, lines 34-35; claim limitation 2 and 10).

Gonzalgo et al., while employing radioactively labeled dNTPs in the amplification step, do not employ fluorescently labeled dNTPs.

Gonzalgo et al. do not employ the differentially labeled fluorescently labeled dNTPs comprising cy3 and cy5.

Yurov et al. disclose the use of multicolor fluorescent detection via use of cyanine dyes, more specifically cy3 and cy5 (page 391, 1st column).

It would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made to modify the teachings of Gonzalgo et al. with the teachings and suggestions of Yurov et al. to arrive at the invention as claimed for the following reasons.

Although Gonzalgo et al. employ radioactively labeled dNTPs and not fluorescently labeled dNTPs, particularly cy3 and cy5 labeled dNTPs, Gonzalgo et al. acknowledge alternate ways of labeling nucleotides (i.e. - fluorescent labels; see page 8, lines 30-31).

In addition to this acknowledgment, Yurov et al.disclose an explicit benefit provided by the use of cy3 and cy5 dye over the traditional fluorescent labels:

"Cyanine dyes are also useful as fluorescent labels or biological macromolecules. Cyanine 3 dye provides significantly **brighter** fluorescence than any other fluorophore, including fluorescein..." (page 391, 1st column).

Yurov et al. also disclose the advantage of using cy3 and cy5 dyes for multicolor detection assays (page 391, 2nd column).

Additionally, it is an art-recognized advantage that the use of fluorescent labels are environmentally safer as well as more efficient.

Therefore, one of ordinary skill in the art at the time the invention was made would have been motivated to take the suggestion of Gonzalgo et al. and the advantage offered by Yurov et al. as well as art-recognized advantage of using fluorescent labels over radioactive labels to arrive at the claimed invention. Since the substitution of fluorescently labeled nucleotides have been well established in the art of nucleic acid amplification and detection as evidenced by Davis et al.:

"Amplified sequences can be labeled by, for example, incorporation of a labeled nucleotides (e.g., a fluorescent nucleotides such as Cy3-dUTP or Cy5-dUTP, or a radioactive nucleotide" (Davis et al., at column 19, lines 18-20)

One of ordinary skill in the art would have had a clear expectation of success at substituting the radioactive labeling with fluorescent labels provided by Yurov et al.

Response to Arguments:

Applicants traverse the present rejection. Applicants' traversal is drawn to the independent claim 1, which has been amended to include step (e), reciting the phrase "then, determining from the measured fluorescence the relative number of methylated cytosine bases that were present in the DNA sample prior to step a)," step (a) being drawn to the treatment of genomic DNA sample with a reagent, wherein 5-methylcytosine and cytosine react differently (page 6, bottom, Response). The preferred embodiment of such reagent is a bisulfite solution which converts unmethylated cytosine to uracil (see claim 6).

Applicants contend that Gonzalgo et al. does not relate to a method for determining the relative number of methylated cytosine bases in a DNA sample, but rather, is directed at a method for determining whether or not a cytosine at a particular location is methylated (page 7, bottom paragraph, Response).

This argument is not found persuasive for the following reasons.

The method disclosed by Gonzalgo et al. employs the treatment of a genomic DNA sample with a bisulfite solution, which results in the conversion of unmethylated cytosine residues to uracil residues while not changing the 5'methylated cytosine residues. This step is the same as step (a) of instant claim 1. The resulting DNA sample is then amplified via PCR with primers specific for bisulfite-converted DNA, followed by the electrophoresis of the amplified product. Applicants appear to be of the assumption that this step is what is relied on by the Examiner in meeting the limitation of step (b) and (c). This assumption, however, is not correct. Gonzalgo et al. takes the amplified product (which contains the chemically reacted DNA sample therein), and conducts SnuPE on the amplified product (see Figure 1). The SnuPE involves the hybridization of a primer to the nucleic acids of the amplified product, followed by its extension with at least labeled dCTP and dGTP (see page 8, lines 6-8). By the extension reaction, the products are "amplified," followed by the separation of the amplified products on a denaturing polyacrylamide gel (page 8, lines 31-34) via electrophoresis, thereby fully meeting step (b) which amplifies via use of labeled dCTP and dGTP; step (c) which spatially separates the amplified product (via electrophoresis). Gonzalgo et al. disclose that the relative quantification of the methylation is determined (page 8, lines 29-28). The methylation detection disclosed by Gonzalgo et al. is drawn to the methylation of 5-methylcytosine. Hence, the relative quantification of the methylation would be that of the cytosine, thereby meeting steps (d) and (e) of claim 1.

Applicants contend that the method of Gonzalgo et al. is directed at a method for determining whether or not a cytosine at a particular location is methylated (page 7, bottom paragraph, Response). Applicants' attention is drawn to the section of the disclosure of Gonzalgo et al. which discusses that the methylation "at each CpG site" is determined (page 8, lines 28-29), as well as CpG island (page 7, lines 35-36; page 9, line 32) using multiple SnuPE primers, resulting in the detection of each of methylated cytosines on the CpG island, resulting in the determination of the relative number of methylated cytosine bases. With regard to argument drawn to the method of Gonzalgo et al. being drawn to determining the methylation of cytosine at a particular location, the method as claimed clearly embraces the method disclosed by Gonzalgo et al., with the exception of using fluorescently labeled nucleotides. In

> addition, the instant specification discloses that "specific primers", MRP3 and MDR1 were employed in an amplification step involving the incorporation of Cy5-dCTP, amplifying a specific region (thus a particular location; page 18, example 3).
>
> With regard to the arguments drawn to the claimed method being drawn to determining "relative number of cytosine methylations," (page 8, bottom paragraph, Response) the method of Gonzalgo et al. determines the number of cytosine methylation relative to the unmethylated cytosines on the DNA strand.
>
> Applicants also contend that the method of the instant application can be used in determining the presence and extent of "co-methylation," a biological phenomenon in which a majority of CpG locations within CpG rich regions share the same methylation status (page 8, bottom paragraph). This argument is not found persuasive because the claims are not drawn to this method. In addition, Gonzalgo et al. disclose a multiplex primer strategy (page 7, line 35-36) which allows the determination of many CpG sites.
>
> Therefore, one of ordinary skill in the art at the time the invention was made would have been motivated to combine the teachings of Gonzalgo et al. with the teachings of Yurov et al. in employing fluorescently labeled nucleotides for the well-known advantage of employing biologically safer material as well as employing labels which produce stronger signals, with a reasonable expectation of success, as evidenced by Davis et al., rendering the invention as claimed *prima facie* obvious over the cited references. (Emphasis in original.)

Insofar as the subject rejection pertains to claim 7, the rejection is moot in view of Applicant's cancellation herein of claim 7. Insofar as the subject rejection pertains to claims 1-3, 6, 8-10, 12 and 13, Applicant respectfully traverses the subject rejection. Claims 2-3, 6, 8-10, 12 and 13 depend from claim 1. Claim 1 has been amended herein and now recites "[a] method for the relative quantification of the methylation of cytosine bases in DNA samples, said method comprising the steps of:

a) chemically reacting a genomic DNA sample with a reagent, wherein 5-methylcytosine and cytosine react differently and these thus show a different base pairing behavior in the DNA duplex after the reaction;

b) then, amplifying the chemically reacted DNA sample by PCR, said amplifying step comprising the use of a fluorescently labeled dCTP or dGTP derivative to yield amplified products, whereby several fluorophores are introduced into one amplified product;

c) then, spatially separating the amplified products from each other;

d) then, quantitatively measuring the fluorescence of the separated amplified products; and

e) then, determining from the measured fluorescence the relative number of methylated cytosine bases that were present in the DNA sample prior to step a)."

Claim 1 is distinguishable over Gonzalgo et al. for at least the reason that Gonzalgo et al. describes a method for primer extension by SINGLE nucleotides. The fact that the Gonzalgo method involves an extension by single nucleotides is stressed by the very name of the method developed by Gonzalgo et al.: Ms-SNuPE = methylation sensitive single nucleotide primer extension (see Gonzalgo et al. at page 1, lines 8-9). Therefore, in accordance with Gonzalgo et al., one particular methylation position is analyzed. If the Gonzalgo method is applied for quantification, the methylation degree at this particular position is analyzed. The methylation status of different methylation positions is not taken into account. If several primers are used in a multiplex reaction, several DISTINCT methylation positions are analyzed simultaneously. Thus, the relative methylation rate at several different positions can be determined. The method described by Gonzalgo is an exact method for the analysis and the quantification of distinct methylation positions. However, Gonzalgo does not teach how to determine an overall methylation level.

The above principle is supported by the passages quoted by the Patent Office: "There are several techniques that are able to determine the relative amount of methylation at EACH CpG site..." (Gonzalgo et al., page 8, line 29); "In one method for determining the relative amount of methylation at EACH CpG site..." (Gonzalgo et al., page 8, line 32); "An alternative method for determining the relative amount of methylation at INDIVIDUAL CpG sites..." (Gonzalgo et al., page 8, line 36). (Emphasis added.)

In contrast to Gonzalgo et al., the present invention involves incorporating SEVERAL labeled nucleotides within a PCR amplification. As a consequence, the analysis of single positions is not possible. On the other hand, the present invention uses the total signal arising from all incorporated nucleotides to determine the overall methylation rate in the whole amplificate. As a result, the present method uses the biological principle of co-methylation under which several adjacent CpG positions bear the same methylation status. As a consequence, the present method is less exact than that of Gonzalgo et al., but more sensitive: As the amplificates contain several labeled nucleotides, the signal is enhanced. Therefore, less sample DNA is required to perform an analysis.

This principle is not obvious over Gonzalgo et al. as Gonzalgo et al. teaches away from incorporating several labeled nucleotides. Instead, Gonzalgo et al. teaches a method for the exact analysis of individual CpG positions. A person of ordinary skill in the art would not have tried to introduce several labeled nucleotides into the amplificate. A person of ordinary skill in the art would have expected that the reliability of the method would suffer, as an exact analysis of an individual position would not be possible anymore. However, by introducing several nucleotides and using the enhancing effect and the co-methylation, it is possible to perform a more sensitive

analysis for which less material is required. This would have been a surprising result to a person of ordinary skill in the art.

Yurov et al. and Davis et al. do not cure the above-mentioned deficiencies of Gonzalgo et al.

Accordingly, for at least the above reasons, the subject rejection should be withdrawn.

Claims 4 and 5 stand rejected under 35 U.S.C. 103(a) "as being unpatentable over Gonzalgo et al. (WO 98/56952, published December 17, 1998) in view of Yurov et al. (Human Genetics, 1996, vol. 97, pages 390-398) and in light of Davis et al. (U.S. Patent No. 6,046,002, issued April 4, 2000, filed January 5, 1998), as applied to claims 1-3, 6-10, 12, and 13 above, and further in view of Apffel et al. (U.S. Patent No. 6,379,889 B1, issued April 30, 2002, filed November 4, 1999) and Roche et al. (Biotechnology Progress, 1997, vol. 13, pages 659-668), made in the Office Action mailed on March 1, 2005...for the reasons of record." In support of the rejection, the Patent Office states the following:

> Applicants' arguments presented in the Amendment received on August 24, 2005 have been fully considered but they are not found persuasive for the following reasons.
>
> All of Applicants' arguments are drawn to claim 1 (as amended) and whether said claim is patentable over the art of record. As already discussed above, claim 1 is not patentable over the cited references, and as Applicants lack additional arguments as to why claims 4 and 5 are patentable over the references of record, the present rejection is maintained for the reasons of record.

Applicant respectfully traverses the subject rejection. Claims 4 and 5 depend from claim 1. Claim 1 is patentable over Gonzalgo et al. in view of Yurov et al. and in light of Davis et al. for at least the reasons given above. Apffel et al. and Roche et al. do not cure all of the deficiencies of

Gonzalgo et al., Yurov et al. and Davis et al. Therefore, based at least on their respective dependencies from claim1, claims 4 and 5 are patentable over the applied references.

Accordingly, for at least the above reasons, the subject rejection should be withdrawn.

Claim 11 stands rejected under 35 U.S.C. 103(a) "as being unpatentable over Gonzalgo et al. (WO 98/56952, published December 17, 1998) in view of Yurov et al. (Human Genetics, 1996, vol. 97, pages 390-398) and in light of Davis et al. (U.S. Patent No. 6,046,002, issued April 4, 2000, filed January 5, 1998), as applied to claim 1 above, and further in view of Wang et al. (Science, May 1998, vol. 280, pages 1077-1082), made in the Office Action mailed on March 1, 2005...for the reasons of record." In support of the rejection, the Patent Office states the following:

> Applicants' arguments presented in the Amendment received on August 24, 2005 have been fully considered but they are not found persuasive for the following reasons.
>
> All of Applicants' arguments are drawn to claim 1 (as amended) and whether said claim is patentable over the art of record. As already discussed above, claim 1 is not patentable over the cited references, and as Applicants lack additional arguments as to why claim 11 is patentable over the references of record, the present rejection is maintained for the reasons of record.

Applicant respectfully traverses the subject rejection. Claim 11 depends from claim 1. Claim 1 is patentable over Gonzalgo et al. in view of Yurov et al. and in light of Davis et al. for at least the reasons given above. Wang et al. does not cure all of the deficiencies of Gonzalgo et al., Yurov et al. and Davis et al. Therefore, based at least on its dependency from claim 1, claim 11 is patentable over the applied references.

Claim 14 stands rejected under 35 U.S.C. 103(a) "as being unpatentable over Gonzalgo et al. (WO 98/56952, published December 17, 1998) in view of Yurov et al. (Human Genetics, 1996, vol. 97, pages 390-398) and in light of Davis et al. (U.S. Patent No. 6,046,002, issued April 4, 2000,

filed January 5, 1998), made in the Office Action mailed on March 1, 2005...for the reasons of record." In support of the rejection, the Patent Office states the following:

> Applicants' arguments presented in the Amendment received on August 24, 2005 have been fully considered but they are not found persuasive for the following reasons.
>
> Applicants contend that the Office has failed to give any weight to the fact that "consisting" of is used instead of comprising (page 13, Response). Applicants contend that the Office has to explain why one of ordinary skill in the art would have been motivated to make all of the modifications needed to change the Gonzalgo method to the claimed method, including the elimination of the primer extension step.
>
> It is respectfully pointed out that SnuPE step involves the "extension" of the primer, resulting in the amplification of the template nucleic acids. In addition, so long as the prior art reference does not teach away from the claimed modification, one of ordinary skill in the art would be motivated to make various modifications of the methods so as to arrive at the claimed invention. In other words, a method consisting of steps: a) isolating DNA from a sample; b) hybridizing a target oligonucleotide probe complementary to a target nucleic acid; and c) detecting the presence of the target nucleic acid; would not be unobvious over a prior art reference teaching a method of steps: a) isolating DNA from a sample; b) amplifying a target nucleic acid via primers; c) hybridizing a target oligonucleotide probe complementary to a target nucleic acid; and d) detecting the presence of the target nucleic acid; solely based on the rationale that the method as claimed is "consisting of." Similarly, so long as the prior art reference does not teach away from the claimed method, it would be obvious to one of ordinary skill in the art to combine the teaching steps as necessary to arrive at the claimed invention, render[ing] the invention as claimed obvious over the cited references. (Emphasis in original.)

Applicant respectfully traverses the subject rejection for the reasons of record. In addition, Applicant respectfully submits that the Patent Office's analysis for concluding that claim 14 is obvious is in error because it is predicated upon an improper shifting of the burden to prove prima

facie obviousness from the Patent Office, where such a burden belongs, to the Applicant to prove non-obviousness, where no such burden exists. The proper issue is whether the prior art teaches or suggests modifying the Gonzalgo et al. method to arrive at the claimed method. The Patent Office has improperly framed the issue as to whether the prior art precludes the possibility of modifying the Gonzalgo et al. method to arrive at the claimed method. Applicant respectfully submits that, with the operative issue properly framed, there is no basis for a finding of obviousness.

Accordingly, for at least the above reasons, the subject rejection should be withdrawn.

Claims 1-13 stand provisionally rejected "under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims 1-29 of copending Application No. 10/220,090...for the reasons of record" and stand provisionally rejected "under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims 1-26 of copending Application No. 10/220,896...for the reasons of record."

Applicant respectfully traverses the subject rejections for the reasons of record.

In conclusion, it is respectfully submitted that the present application is now in condition for allowance. Prompt and favorable action is earnestly solicited.

If there are any fees due in connection with the filing of this paper that are not accounted for, the Examiner is authorized to charge the fees to our Deposit Account No. 11-1755. If a fee is required for an extension of time under 37 C.F.R. 1.136 that is not accounted for already, such an extension of time is requested and the fee should also be charged to our Deposit Account.

Respectfully submitted,

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Dated: May 23, 2006

I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to: Mail Stop Amendment, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450 on May 23, 2006

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